

Soil microbial properties and nutrients in pure and mixed Chinese fir plantations

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Abstract: An investigation on soil organic carbon, total N and P, NO_3^- -N, available P, microbial biomass C, N and P, basal respiration and metabolic quotients ($q\text{CO}_2$) was conducted to compare differences in soil microbial properties and nutrients between 15-year-old pure Chinese fir (*Cunninghamia lanceolata*) and two mixed Chinese fir plantations (mixed plantations with *Alnus cremastogyne*, mixed plantations with *Kalopanax septemlobus*) at Huitong Experimental Station of Forest Ecology (26°45'N latitude and 109°30'E longitude), Chinese Academy of Sciences in May, 2005. Results showed that the concentrations of soil organic carbon, total N, NO_3^- -N, total P and available P in mixed plantations were higher than that in pure plantation. Soil microbial biomass N in two mixed plantations was averagely higher 69% and 61% than that in pure plantation at the 0–10 cm and 10–20 cm soil depth, respectively. Soil microbial biomass C, P and basal respiration in mixed plantations were higher 11%, 14% and 4% at the 0–10 cm soil depth and 6%, 3% and 3% at the 10–20 cm soil depth compared with pure plantation. However, soil microbial C: N ratio and $q\text{CO}_2$ were averagely lower 34% and 4% in mixed plantations than pure plantation. Additionally, there was a closer relation between soil microbial biomass and soil nutrients than between basal respiration, microbial C: N ratio and $q\text{CO}_2$ and soil nutrients. In conclusion, introduction of broad-leaved tree species into pure coniferous plantation improved soil microbial properties and soil fertility, and can be helpful to restore degraded forest soil.

Keywords: Chinese fir; mixed plantation; soil microbial biomass; soil nutrient

Introduction

Globally, large-scale pure plantations have been established in the world to meet the world's growing demands for timber, fuel material and other forest products since the 20th century, especially within the recent three decades (Evans 1998). In subtropical regions, the native broad-leaved forests have almost been destructed by human activities, and then gradually replaced by pure coniferous plantations such as Chinese fir (*Cunninghamia lanceolata*) and pine (*Pinus massoniana*). At present, more than 1.21×10^7 ha Chinese fir plantations have been established in China, accounting for about 24% of the total plantation area (Chen and Wang 2004). However, some researchers observed that the system of successive stands of Chinese fir could lead to

soil degradation and yield decline and proposed that mixtures with broad-leaved tree species should be planted to avoid soil degradation and improve soil fertility (Chen et al. 1990; Ding and Chen 1995). Wang et al. (2005; 2006) reported soil microbial biomass in pure coniferous plantations was significantly lower than that in native broad-leaved forest. However, few direct comparisons of microbiological properties under pure and mixed plantations of Chinese fir have been made.

Tree species may influence microbial processes associating with carbon and nitrogen cycling in forest ecosystem (Morisada et al. 2004; Xu and Inubushi, 2004) due to differences in quality, quantity and decomposition rates of litters, which in turn may affect the status of readily substances for microbial growth in soils. Tree species have an impact on soil nutrients and microbial community structure, in turn, which can affect soil microbial biomass and microbial efficiency in C utilization. Microbial metabolic quotient ($q\text{CO}_2$), described by Odum (1969) as the ratio of respired C to microbial biomass C, is considered as an index for evaluating C utilization efficiency of soil microbes measured during a short-term incubation (Wardle and Ghani 1995). However, our knowledge is limited to the effect of tree species on microbial biomass and metabolic status in subtropical forest soils (Wang and Wang 2007).

In this study, mineral soils under one pure and two mixed Chinese fir plantation stands were collected at Huitong Experimental Station of Forest Ecology, Chinese Academy of Sciences to study the effects of broad-leaved tree species introduced into

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pure coniferous plantation on soil microbial properties. Additionally, soil nutrients were also investigated under three Chinese fir plantations in this study. The results can improve the understanding of effect of broad-leaved trees in coniferous plantation on soil fertility and restoration of degraded soil in forest ecosystems.

Materials and methods

Site description

The study site was located at Huitong Experimental Station of Forest Ecology (26°45'N latitude and 109°30'E longitude), Chinese Academy of Sciences, in Hunan Province. This Experimental Station lies at the transition from the Yuan-Gui Plateau to the hills on the southern bank of Yangtze River and belongs to the upper reach of Yuan River, a tributary of Yangtze. The altitude

ranges from 300 to 1100 m above the sea level. This region has a humid mid-subtropical monsoon climate with average annual temperature of 16.5°C. The annual precipitation ranges from 1200 to 1400 mm, most of the rain falling between April and August, and the annual evaporation from 1100 to 1300 mm. The soil of the experimental field is Oxisols according to U.S. Soil Classification (Wang and Wang 2007).

One pure Chinese fir plantation (PCF) and two mixed plantations with *Alnus cremastogyne* (MCA) and *Kalopanax septemlobus* (MCK) were established in early spring of 1990 after clear-cutting of a first generation of Chinese fir forest in autumn of 1989 (Table 1). The three plantations were in close proximity and had similar soil attributes before establishment. Unplanted buffer strips, each 10-m wide, were left between the stands to reduce the effects of shading, and a protection area was planted with trees around the experimental site. Three replicated plots (10 m × 20 m) were selected in each of the three forest stands.

Table 1. General conditions of experimental forest stands

Plantation type	Tree species	Stand age (year)	Density (stem·ha ⁻¹)	DBH (cm)	Height (m)	Elevation (m)
pure Chinese fir plantation	<i>Cunninghamia lanceolata</i>	15	1498	19.7	18.5	521
mixed plantations with	<i>Cunninghamia lanceolata</i>	15	1201	20.5	19.6	534
<i>Alnus cremastogyne</i>	<i>Alnus cremastogyne</i>	15	267	18.9	16.4	534
mixed plantations with	<i>Cunninghamia lanceolata</i>	15	1179	20.3	19.3	548
<i>Kalopanax septemlobus</i>	<i>Kalopanax septemlobus</i>	15	283	18.1	17.8	548

Soil sampling and analysis

Soil samples were collected at the depth of 0–10 cm and 10–20 cm from each plot. Each composite sample consisted of ten randomly collected sub-samples at the same depth in each plot. In other words, six composite samples were taken in each forest stand at two soil depths. Therefore, 18 composite samples in all were taken in this study. Each sample was divided into two parts. One was sieved through a 2-mm mesh immediately and then stored at 4°C until analysis for microbial biomass and basal respiration for up to three days. The other part of soil was subsequently air-dried, and then ground.

Soil organic C (SOC) and total nitrogen was determined using Vario-MAX C/N auto-analyzer (made in Germany; Elementar). The combustion process converts any soil C into CO₂ gases. NH₄⁺-N and NO₃⁻-N were extracted with 1 mol·L⁻¹ KCl solution, and then the filtered solution was measured by colorimetry. Total phosphorus (P) was analyzed using the molybdate blue method after digestion with sulfuric acid and perchloric acid, while available P was measured with Olsen method (1982). Fresh soil pH was measured using a portable pH meter. Chloroform fumigation-extraction method was used to estimate soil microbial biomass C (MBC), N (MBN) and P (MBP). MBC and MBN were determined in fresh soil by chloroform fumigation-extraction method using 0.5 mol·L⁻¹ K₂SO₄ as extracting solution. In brief, duplicate samples (25 g dry weight) were fumigated with chloroform and then extracted with 100 ml 0.5 mol·L⁻¹ K₂SO₄ for 0.5 h on an end-over-end shaker. The suspended samples were filtered through quantificational filter paper.

Similar sets of non-fumigated sample were extracted in the same way. The amounts of total C and N in the extracts of the non-fumigated and fumigated soils were determined on a Phoenix-8000 TOC and FIA-Star 5000 auto-analyzer, respectively. Soil microbial biomass C and N were calculated using an *Ec* factor of 2.2 (Wu et al. 1990) and an *En* factor of 2.2 (Brookes et al. 1985). MBP was determined by Chloroform fumigation-extraction method using 0.5 M NaHCO₃ as extracting solution (Brookes et al. 1982), and the content of P was measured using the molybdate blue method. Basal respiration was determined by measuring CO₂ evolution. The field-moist soils (equivalent to 100 g dry weight) were placed in a 1000-mL air-tight glass flasks and aerobically incubated at 28°C for 24 h. Vials containing a 20-mL 0.1 mol·L⁻¹ NaOH were placed inside the flasks and the CO₂ evolved from soil was trapped. The residual NaOH was titrated with 0.05 mol·L⁻¹ HCl to phenolphthalein endpoint. Carbon dioxide evolved was calculated from the difference in normality between NaOH blanks and samples (Chen and Xu 2004).

Statistical analysis

Differences in soil nutrients and microbial properties between pure and mixed Chinese fir plantations were performed using an analysis of variance (ANOVA) with SPSS 11.5 for Windows. The relationships between soil microbial properties and nutrients were analyzed by using Microsoft Excel 2003.

Results

Soil nutrient

The results of soil nutrients under different plantations in this study showed that the concentrations of soil nutrients were slightly higher under mixed plantations than that under pure

plantation (Table 2). The difference in soil nutrients except for total N and available P between pure and mixed plantations was not significant. As for 0–10 cm soil depth, total N in mixed plantations were significantly higher than that in pure plantation. Available P was significantly higher in MCK than in PCF only at the depth of 10–20 cm. In addition, pH in mixed plantations was significantly higher compared to pure plantation.

Table 2. Concentrations of soil nutrients under pure and mixed Chinese fir plantations

Plantation type	Depth (cm)	SOC (g·kg ⁻¹)	Total N (g·kg ⁻¹)	NO ₃ ⁻ -N (mg·kg ⁻¹)	NH ₄ ⁺ -N (mg·kg ⁻¹)	Total P (g·kg ⁻¹)	Available P (mg·kg ⁻¹)	pH
PCF	0-10	11.4 (0.6)a	1.13 (0.08)b	8.48 (1.94)a	8.75 (0.38)a	0.157 (0.022)a	7.73 (1.87)a	3.94 (0.10)b
MCA		11.7 (0.8)a	1.34 (0.04)a	12.07 (1.60)b	9.77 (0.78)a	0.169 (0.009)a	8.62 (0.69)a	4.24 (0.03)a
MCK		12.3 (1.0)a	1.31 (0.06)a	8.93 (0.28)a	9.12 (0.32)a	0.181 (0.034)a	8.06 (0.29)a	4.21 (0.01)a
PCF	10-20	9.3 (3.8)a	1.03 (0.24)a	5.84 (1.52)a	8.09 (0.63)a	0.150 (0.035)a	4.12 (0.30)b	3.83 (0.05)b
MCA		9.9 (2.0)a	1.16 (0.11)a	6.33 (1.37)a	9.71 (0.68)a	0.159 (0.011)a	5.36 (0.42)ab	4.17 (0.03)a
MCK		11.3 (0.5)a	1.27 (0.10)a	6.66 (0.41)a	9.05 (1.01)a	0.154 (0.018)a	4.93 (0.44)a	4.15 (0.05)a

Note: Data in the parenthesis are standard deviations. Values with different letters at the same column in the same soil depth are significant at the level of 0.05. PCF, pure Chinese fir plantation; MCA, mixed plantations with *Alnus cremastogyne*; MCK, mixed plantations with *Kalopanax septemlobus*; SOC, Soil organic C.

Soil microbial properties

Soil microbial properties of Chinese fir plantations were shown in Table 3. In the present study, the differences of these parameters between pure and mixed Chinese fir plantations were not significant ($P > 0.05$), although introduction of broad-leaved

trees increased the values of MBC, MBP and basal respiration, and decreased $q\text{CO}_2$. Mixed plantations had a higher MBN content and lower ratio of microbial C to N as compared to pure plantation. The differences in MBN and ratio of microbial C to N between pure and mixed plantations were significant.

Table 3. Soil microbial properties of pure and mixed Chinese fir plantations

Plantation type	Depth (cm)	MBC (mg·kg ⁻¹)	MBN (mg·kg ⁻¹)	MBP (mg·kg ⁻¹)	Basal respiration (mg CO ₂ ·g ⁻¹ dry weight soil d ⁻¹)	$q\text{CO}_2$ (g CO ₂ -C kg ⁻¹ MBC h ⁻¹)	Microbial C: N ratio
PCF	0-10	254 (35)a	21.0 (2.0)b	14.9 (2.6)a	42.3 (4.3)a	1.90 (0.51)a	12.15 1.77)a
MCA		292 (32)a	37.6 (4.4)a	16.9 (1.1)a	44.9 (2.0)a	1.76 (0.16)a	7.82 (1.05)b
MCK		272 (37)a	33.2 (6.1)a	17.3 (2.1)a	43.0 (3.5)a	1.85 (0.24)a	8.25 (0.91)b
PCF	10-20	213 (12)a	15.7 (1.5)b	14.9 (2.6)a	30.4 (1.4)a	1.62 (0.08)a	13.66 1.74)a
MCA		228 (23)a	26.1 (2.9)a	16.3 (2.5)a	30.7 (2.5)a	1.52 (0.13)a	8.82 (1.34)b
MCK		222 (14)a	24.5 (3.2)a	14.4 (2.4)a	31.9 (1.3)a	1.62 (0.12)a	9.22 (1.85)b

Data in the parenthesis are standard deviations. Values with different letters at the same column in the same soil depth are significant at the level of 0.05. PCF, pure Chinese fir plantation; MCA, mixed plantations with *Alnus cremastogyne*; MCK, mixed plantations with *Kalopanax septemlobus*; MBC, microbial biomass C; MBN, microbial biomass N; MBP, microbial biomass N.

Relationships between soil microbial properties to nutrients

A matrix of the correlation coefficients between soil microbial properties to nutrients under all studied plantations was shown in Table 4. There were significantly positive relations between MBC and SOC, NO₃⁻-N and available P in the forest soils ($P < 0.05$). The relationships between MBN and soil nutrients except for total P were significant. MBP was strongly correlated with soil nutrients except for SOC and pH. The ratio of microbial C to N was significantly negatively correlated with total N and pH. However, there was no significant correlation between $q\text{CO}_2$ and soil nutrients in this study.

Discussions

Soil nutrients, especially available nutrients are closely linked to plant productivity and often used as indicators of soil fertility (Moria 2002). In the present study, the results showed that the introduction of broad-leaved tree species into pure coniferous forests increased the contents of SOC and soil nutrients (Table 2), although the differences in some parameters were not significant. Total N concentration in pure plantation drastically decreased, indicating that N may be a limiting factor for Chinese fir growth. Wang et al. (2000) had observed that soil organic C, NO₃⁻-N and available P levels in pure plantation were lower than those of mixed plantations. Litter is an important source of soil nutrients

and its decompositions contribute to the regulation of nutrient cycling as well as soil fertility (Fenn 1991). Some researches had suggested that amount of litterfall in mixed plantations was much higher than that in pure coniferous plantations as other conditions such as site quality and climate were similar (Chen and

Wang 2004). Litter decomposition was also controlled by litter quality, and coniferous litter quality would be improved by adding broad-leaved tree species. Thus, nutrient returned to soil through litter were higher in mixed plantations than in pure plantation.

Table 4. Pearson correlation coefficients of soil nutrients and microbial properties under different Chinese fir plantations ($n = 18$)

	MBC	MBN	MBP	Basal respiration	$q\text{CO}_2$	Microbial C: N ratio
SOC	0.466*	0.531*	0.277	0.458*	0.134	-0.418
Total N	0.380	0.660**	0.498*	0.303	0.003	-0.596**
NO_3^- -N	0.783**	0.663**	0.542*	0.687**	0.092	-0.279
NH_4^+ -N	0.342	0.484*	0.468*	0.220	-0.079	-0.436
Total P	0.384	0.232	0.884**	0.187	-0.178	-0.026
Available P	0.756**	0.643**	0.565**	0.749**	0.196	-0.340
pH	0.367	0.809**	0.377	0.278	-0.216	-0.868**

*, ** Correlation is significant at the 0.05 and 0.01 level, respectively. SOC, Soil organic C; MBC, microbial biomass C; MBN, microbial biomass N; MBP, microbial biomass N.

Soil microbial biomass, the most active fraction of soil organic matter, plays an important role in nutrient retention and soil fertility in terrestrial ecosystems, although it only presents a relatively small standing stock of nutrients compared to soil organic matter. In this study, soil microbial biomass C, N and P in mixed plantations were slightly higher than that in pure plantation. The increase in microbial biomass may be due to high quality and amount of litter under mixed plantation.

Variation of microbial C: N ratios indicated that microbial biomass changed in a way due to the change in composition of soil microflora. A higher ratio usually suggests a high proportion of fungi compared to bacteria, which C: N ratio of fungi is in the range of 7–12 and that of bacteria often ranges from 3 to 6 (Anderson and Domsch 1980). The ratio of microbial C to N in mixed plantation was significantly lower than that in pure plantations. This indicated that the proportion of bacteria increased in mixed plantations. Some studies showed that the amount of bacteria was higher in broad-leaved forests compared to pure Chinese fir plantation (Hu et al. 2005). There was a significantly negative correlation between microbial C: N ratio and pH in Chinese fir plantations (Table 4), suggesting that bacteria may occur in soils with lower acidity than fungi does.

Microbial metabolic quotient, namely $q\text{CO}_2$, is termed as the ratio of respired C to biomass C. It is considered as an index for evaluating substrate utilization efficiency of soil microbial community (Insam 1990) and a much more sensitive indicator of stress, and used to assess the process of soil development or degradation (Killham and Firestone 1984; Insam et al. 1989). More efficiently the micro-organisms function, greater the fraction of substrate C is incorporated into biomass and less C per unit biomass is lost through respiration, which results in a low metabolic quotient (Behera and Sahani 2003). Thus, the higher metabolic quotient ($q\text{CO}_2$) in pure plantations reflects a decrease in the efficiency of substrate utilization by soil microbial community. The low microbial C utilization in pure Chinese fir plantation soil was consistent with the results of Huang et al. (2004). There was a negative correlation between the $q\text{CO}_2$ and soil pH in Chinese fir plantations ($r = 0.216$, $n = 18$), and the soils under

mixed plantations had a higher pH values than that under pure plantation (Table 2). This indicated that the increase in soil pH was possibly associated with the decrease in $q\text{CO}_2$. Xu et al. (2006) reported that microbial communities released more CO_2 -C per unit biomass and per unit time under pine forest soil conditions than communities at a more neutral pH range. Therefore, the enhancement of the $q\text{CO}_2$ is caused by low acidity, and considered as indicative of stress in microbial communities and their activities.

Conclusions

Introduction of broad-leaved tree species into pure coniferous plantation enhanced concentrations of soil organic carbon, total N, NO_3^- -N, total P and available P. Soil microbial properties were also improved by introduction of broad-leaved tree, and microbial biomass N increased 65% in the whole soil, but microbial biomass C and P only increased about 8% averagely. Therefore, introduction of broad-leaved trees into pure coniferous plantation can improve soil fertility and be helpful to restore degraded forest soil.

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